Evaluation of New Immunohistochemical Approaches for the Study of Kidney Tumors in Geriatric

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Abstract
Kidney malignancies are one of the most deadly genitourinary tumors. It is more common in men and is often seen in people aged 70-60 years. The incidence of kidney cancer seems to be increasing. One reason for this may be the fact that imaging techniques such as computed tomography scans are more commonly used. These tests may lead to the accidental detection of more kidney cancers. Fortunately, kidney cancer is often detected in the early stages, when the cancer is small and confined to the kidney. The objective of this study was the development of new diagnostic immunohistochemical methods. Clinical examination material of 134 people, 94 (70%) men, and 40 (30%) women were used in this study. Immunohistochemical staining of tryptase in compliance with the requirements was carried out using Anti-Mast Cell Tryptase antibodies. Goat anti-mouse antibodies #AS-M1-HRP were used as secondary antibodies, visualized with ImmPACTTM DAB Peroxidase Substrate Kit (#SK-4105) according to the manufacturer's instructions. The nuclei were counterstained with Mayer's hematoxylin, and the sections were embedded in a permanent mounting medium. The immunohistochemical study showed an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma compared with the control group. The number of mast cells with tryptase expression directly in the tumor was significantly less than the peritumoral localization. A similar pattern was observed for chymase-positive mast cells the content of the tumor was more than ten times higher than the intratumoral arrangement. The histological and immunological characteristics
did not differ in different age groups. The immunohistochemical method of research in the diagnosis of renal tumors plays an important diagnostic and prognostic value. It can assist pathologists in difficult and ambiguous cases to correctly diagnose renal tumors. This will make it possible to prescribe the correct treatment and predict the course of malignant tumor growth in patients.

**Keywords**: Kidneys, metastases, oncurology, trace elements

### 1. Introduction

Kidney tumors are one of the 10 most dangerous diseases, which is more common between the ages of 50 and 70 and is one of the most common cancers (1, 2). It accounts for 2 to 4% of malignant cancers. In urology, it is one of the three most important diseases. The prevalence of the kidney tumors are significantly higher in men. Smoking plays a big role in causing kidney tumors. The first sign is usually the presence of blood in the urine, at which time the doctor finds a tumor by performing an ultrasound (3, 4). There are cancer cells in all humans that die spontaneously or lose their destructive effect under the influence of the immune system (5-7). A programmed cell death (apoptosis) plays an important role in the spontaneous death of cancer cells (8, 9).

In recent years, both in Russia and globally, there has been a tendency to an increase in the incidence of malignant tumors, including kidneys, which attracts the attention of various specialists (urologists, oncologists, pathomorphologists). Malignant kidney tumors rank 10th in the cancer registry among all neoplasms. According to the literature, it occurs in patients aged from 30 years and older (10, 11). Men suffer almost 2 times more often than women (10-12). In recent years, renal tumors (especially malignant ones) have been more often diagnosed late, when the pathological process already has signs of aggressive growth: tumor growth into the vessels, which further leads to the formation of metastases and shortens the survival time of patients (13, 14).

The cause of most kidney cancers is unknown, according to medical findings the genetic material/DNA in some kidney cells is mutated, which causes kidney cancer. Changes in this substance cause cells to grow and divide rapidly. The accumulation of these abnormal cells causes a tumor that can involve nearby organs in addition to the kidney, which is called metastasizing the involvement of organs farther away from the kidney (15-17).

Kidney cancer has different types such as Renal cell carcinoma, Urothelial carcinoma, Sarcoma, Wilms tumor, and Lymphoma (18, 19) and the prognosis of malignant renal tumors, as well as in tumors of other localizations, depends on the presence or absence of regional and distant metastases, on the stage and histological variant of the disease, which cannot be differentiated without morphological examination (12-14).

About one percent of all oral cancers are metastases from primary tumors that occur in other parts of the body. The most common kidney tumor is considered to be a malignant epithelial tumor – renal
cell carcinoma (RCC) - 80-90% of all kidney tumors. RCC has a large number of histological variants of the structure: the most common is the light-cell variant; the papillary, chromophobic and other variants are less common (20-22). Immunohistochemical study is currently considered one of the most informative, as it allows the most accurate determination of both the histogenesis of the tumor and to establish the degree of its differentiation (20, 22). At the present stage of development of morphology, there are no difficulties in obtaining antibodies to almost any antigen. By studying specific molecules, immunohistochemistry provides information about the functional state of the cell, its interaction with the microenvironment, to establish the phenotype and belonging of the cell to a particular tissue.

The epithelial nature of the kidney tumor can be confirmed by antibodies such as the epidermal growth factor receptor (EGFR) and AMACR. AMACR is a specific marker for detecting prostate cancer but it also gives a response to malignant epithelial tumors of other localizations (14, 23, 24). Antibodies of proliferation and apoptosis help to establish tumor differentiation P53, P63, Ki-67, PCNA: the more intense the expression of proliferation markers is, the more aggressively the tumor behaves and, accordingly, has a lower differentiation. Accordingly, for each kidney tumor, as well as for each variant of RCC, there is its own original combination and intensity of staining with antibodies. However, there is still no single scheme of differentiation of various variants of kidney tumor growth.

In this regard, the objective of this study was the development of new diagnostic immunohistochemical methods.

2. Materials and Methods
The research was based on the material obtained in 2015-2020 at Belgorod Oncological Dispensary, St. Joseph Belgorod Regional Clinical Hospital, and Belgorod Pathologic-anatomical Bureau. The study of the material, analysis and processing of the results obtained were carried out at the Department of Pathology and at the Scientific, Educational and Innovation Center of Nanstructured Materials and Nanotechnologies of Belgorod State University.

The study involved examination of 134 people, of which 94 (70%) were men, and 40 (30%) were women. Groups were formed according to age and nosological criteria (Table 1).
Table 1. Patients with pathology of the kidney

<table>
<thead>
<tr>
<th>Pathology of the kidneys, bladder (n=114)</th>
<th>Control (n=20)</th>
<th>Middle age (40-49 years)</th>
<th>n=10</th>
<th>Elderly age (60-83 years)</th>
<th>n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney cysts (n=22)</td>
<td>Middle age (41-55 years)</td>
<td>n=10</td>
<td>Elderly age (60-78 years)</td>
<td>n=12</td>
<td></td>
</tr>
<tr>
<td>Kidney cancer (women 40 and men 52) n=92</td>
<td>Middle age (40-55 years) (n=43)</td>
<td>I stage (T1 N0 M0)</td>
<td>n=10</td>
<td>II stage (T1:T2 N0M0)</td>
<td>n=12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III stage (T1:T3 N1:N2)</td>
<td>n=13</td>
<td>IV stage (T1:T3 N1: N2 M1)</td>
<td>n=8</td>
</tr>
<tr>
<td></td>
<td>Elderly age (61-79 years) (n=49)</td>
<td>I stage (T1 N0 M0)</td>
<td>n=10</td>
<td>II stage (T1:T2 N0M0)</td>
<td>n=18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III stage (T1:T3 N1:N2)</td>
<td>n=16</td>
<td>IV stage (T1:T3 N1: N2 M1)</td>
<td>n=5</td>
</tr>
</tbody>
</table>

All the subjects had neither exacerbation of chronic diseases, nor severe concomitant somatic pathology. Also, the patients of the control groups did not present urological complaints, and purposefully did not turn to specialists of this profile.

For histological examination under light microscopy, specimens were cut out from various parts of the kidneys, fixed, embedded in paraffin and sectioned on a microtome, followed by staining with hematoxylin and eosin, then examined and photographed in a Topic-T light Ceti microscope. The work analyzed the expression of tryptase and chymase in mast cells associated with kidney cancer. The group of patients with kidney cancer consisted of 5 patients. The control group consisted of 6 patients. Immunohistochemical staining of tryptase in compliance with the necessary requirements (14) was carried out using Anti-Mast Cell Tryptase antibodies (clone AA1, #ab2378, dilution 1:3000), chymase – Anti-Mast Cell Chymase antibodies (clone CC1, #ab2377, 1:2000). Goat anti-mouse
antibodies #AS-M1-HRP were used as secondary antibodies, visualized with ImmPACTTM DAB Peroxidase Substrat Kit (#SK-4105) according to the manufacturer's instructions. The nuclei were counterstained with Mayer's hematoxylin, and the sections were embedded in a permanent mounting medium.

To assess the expression profile of kidney mast cell proteases, double immunolabeling was used (14). Simultaneous staining was performed with AntiMast Cell Tryptase antibody EPR9522] (ab151757, dilution 1:1000) and Anti-Mast Cell Chymase antibody (clone CC1, #ab2377, 1:1000) in accordance with the standard protocol. To detect primary antibodies, Goat Anti-Mouse IgG H&L (ab97035) and Goat Anti-Rabbit IgG H&L (ab150077) were used, conjugated with Cy3 and Alexa Fluor 488 fluorochromes, respectively. Next, the nuclei were counterstained with DAPI (5 μg/ml PBS; Sigma) for 15 seconds, washed with phosphate buffer, and the sections were embedded in an anti-fluorescent mounting medium.

Sections were examined using a ZEISS Axio Imager.A2 microscope with an image recording system including a Camera Axiocam 506 color digital camera and a Camera Axiocam 503 mono monochrome camera. The images obtained were processed using ZEN 2.3 software (Carl Zeiss, Germany). The volume of the kidney mast cell population was determined in conventional visual fields using a ×40 lens, the number of which was at least 50 to obtain a representative data array. After the performed planimetric analysis, to facilitate the perception of the obtained digital array, the results were adapted to a tissue area of 1 mm².

To identify somatic pathology, diagnostic measures were carried out: collection of complaints and anamnesis with a targeted survey of systems and organs, physical examination, as well as laboratory and instrumental research methods: general blood test, general biochemical blood test, general urine analysis, electrocardiogram registration, study respiratory function, chest x-ray.

In the presence of kidney pathology in patients, in order to establish and clarify the diagnosis, laboratory and instrumental examination was carried out, including a comprehensive ultrasound examination of internal organs, lymph nodes, microbiological examination of urine, scintigraphy of the bones of the skeleton, if necessary, computed tomography. The study included patients with histological verification of the disease.

3. Results and Discussion

Histological examination of the obtained kidney biopsies showed that the majority (93%) of the tumor was represented by clear cell carcinoma. It was a malignant tumor with a thin vasculature, consisting of cells with light or eosinophilic cytoplasm. It was predominantly solitary and located in the renal cortex (23, 24). Macroscopically, for the most part, it was yellow, in the form of a node with clear boundaries and a pseudocapsule. Often it was characterized by secondary changes in the form of foci
of necrosis, hemorrhages, calcifications (19). Invasion of the tumor into the perineal tissue and/or in growth into the renal vein was observed in about 41% of cases, it is similar to the previously reported research (19-22). The rest of the cases (7%) were papillary cancer. In half of the cases, a multifocal variant was observed, the multifocal variant was more common in men; the average age of the patients was 53-65 years, this findings were in agreement with previously published works (18-20). Histologically, for the most part, it contained papillary structures of small cells with scanty cytoplasm and small nuclei. In two cases, larger cells with well-defined eosinophilic cytoplasm, large nuclei, and prominent nucleoli were observed. The histological picture did not differ in different age groups. The immunohistochemical study we showed an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma compared with the control group, this is in agreement with previous published works (21-24). At the same time, the activity of degranulation of proteases by mast cells increases, which was morphologically manifested by the expansion of protease-positive pericellular staining of structures of the tumor microenvironment. At the same time, the number of mast cells with tryptase expression directly in the tumor was significantly less than in the peritumorous localization (20-24). A similar pattern was observed for chymase-positive mast cells, the content of which around the tumor was more than 10 times higher than in the intratumoral arrangement. A detected protease profile indicates a significant increase in the expression of chymase in the mast cell population, with the highest intensity in the peritumorous region, which was reflected in an increase in the number of mast cells with simultaneous expression of proteases. The data obtained indicate that the development of kidney cancer in general is accompanied by an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma compared with the indicators of the control comparison group (Figure 1). The immunological characteristics in different age groups did not differ.
Thus, the immunohistochemical method of research in the diagnosis of renal tumors plays an important diagnostic and prognostic value. It can assist pathologists in difficult and ambiguous cases to correctly diagnose renal tumors. This will make it possible to prescribe the correct treatment and predict the course of malignant tumor growth in patients.

The development of kidney cancer is accompanied by an increase in the population of tryptase- and chymase-positive mast cells in the organ. Evaluation of the expression level of tryptase and chymase
reflects the invasive ability of tumor tissue and can be a pathomorphological criterion for the aggressiveness of kidney cancer. Determination of the prognostic role of the number of mast cells in the kidney requires considering their histotopographic localization in the organ. The histological and immunological characteristics did not differ in different age groups.

References


